

DOUGLAS et al.
Appl. No. 10/554,266
July 17, 2009

AMENDMENTS TO THE TITLE:

Please change the title to read as follows:

“Analytical Method Involving Detection Of An Exciplex”.

AMENDMENTS TO THE SPECIFICATION:

At page 18, between lines 23 and 24, insert the following section heading

Brief Description of the Drawings

Delete the paragraph beginning at page 18, line 24 and insert the following new paragraphs in lieu therof.

Figures 1A-1K illustrate the chemical structures of various hybridisation constructs as investigated in the following Examples. All of the constructs shown in Fig 1 have the same nucleic acid sequences for the target and probes as set out in Example 1 for SP-1, SP-19 and SP-34.

Figure 2 illustrates the emission spectra of SP-1 in Tris buffer (10 mM TRIS, pH 8.5, 0.1 M NaCl) in the presence of hexafluoro-2-propanol additive (50%) compared with 80% TFE additive.

Figure 3 illustrates the emission of spectra of SP-1 in Tris buffer (10 mM TRIS, pH 8.5, 0.1 M NaCl) in the presence of tetrafluoro-1-propanol additive (50% and 70%) compared with 80% TFE additive.

Figure 4 illustrates the emission spectra of SP-1 in Tris buffer (10 mM TRIS, pH 8.5, 0.1 M NaCl) in the presence of ethylene glycol (50% and 70%) compared with 80% TFE additive.

Figure 5 illustrates the emission spectra of SP-19 in Tris buffer (10 mM TRIS, pH 8.5, 0.1 M NaCl) in the presence of ethylene glycol additive (50% and 70%).

Figure 6 illustrates the emission spectra of SP-34 in Tris buffer (10 mM TRIS, pH 8.5, 0.1 M NaCl) in the presence of ethylene glycol dimethyl ether additive (80%) compared with 80% TFE additive.

Figure 7 illustrates the emission spectra of SP-34 in Tris buffer (10 mM TRIS, pH 8.5, 0.1 M NaCl) in the presence of ethylene glycol dimethylether additive (80%).

Figure 8 illustrates the emission of spectra of SP-17 in Tris buffer in the presence of 80% TFE additive.

Figure 9 illustrates the emission of spectra of SP-18 in Tris buffer in the presence of 80% TFE additive.

Figure 10 illustrates the emission of spectra of SP-19 in Tris buffer in the presence of 80% TFE additive.

Figure 11 illustrates a study of the exciplex emission of SP-19 in 80% TFE at temperatures of 15°C, 20°C, 25°C, 40°C with cooling back to 10°C.

Figure 12A illustrates the emission spectra of SP-19 and Figure 12B displays the emission spectra of SP-23 both recorded in Tris buffer containing 80% v/v TFE so as to provide a comparison of the relative effectiveness of perylene and pyrene as the acceptor partner for an exciplex with the same donor partner.

Figure 13 illustrates the emission of spectra of SP-1, SP-4 and SP-19 in Tris buffer in the presence of 80% TFE additive.

Figure 14 illustrates the emission of spectra of SP-18 and SP-20 in Tris buffer in the presence of 80% TFE additive.

Figure 15A illustrates the emission spectra for the RNA-based SP-19 exciplex system comparing the RNA target to the equivalent DNA target system (SP-19). Figure 15B illustrates the emission spectra of 5-pyrene-bearing oligo (ON1-5'pyrene) and the full RNA-BASED SP-19 system in Tris buffer at 10°C. Figure 15C illustrates emission spectra of RNA-based SP-19 IN Tris buffer at various TFE concentrations. Figure 15D Illustrates the emission spectra of the RNA-BASED SP-19 system in 70% TFE/Tris buffer.

Figure 16 illustrates melting curves for RNA-based SP-19 in 72% TFE/Tris buffer.

Figure 17 illustrates emission spectra for the SP-19 exciplex system with mismatch targets before heating.

Figure 18 illustrates emission spectra for SP-19 exciplex system with insertion targets before heating.

Figures 19A and 19B provide a graphical comparison of the data shown in Tables 2 and 3, respectively.

Figure 20 illustrates the emission of spectra of SP-25 in Tris buffer in the presence of 80% TFE additive and in the absence of TFE additive.

Figure 21 illustrates the emission of spectra of SP-26 in Tris buffer in the presence of 80% TFE additive.

Figure 22 illustrates the emission spectra of ON1-5' pyrene and ON2-3' Naphthalene in the presence of target strand in Tris buffer and in the presence of sulfolane.

Figure 23 illustrates the emission spectra for the SP-19 system in 80% TFE/Tris buffer in the presence of 0.1 and 1.5 M betaine and in the absence thereof.

Figure 24 illustrates the emission spectra for the SP-19 system in 80% TFE/Tris buffer in the presence of 0.15 and 0.5 M sulfolane and in the absence thereof.

Figure 25 illustrates the emission spectra for the SP-19 system in 80% TFE/Tris buffer in the presence of 0.6 and 1.1 M Methylsulfone and in the absence thereof.

Figure 26 illustrates the emission spectra for the SP-19 system in 80% TFE/Tris buffer in the presence of 1.41M DMSO and in the absence thereof.

Figure 27 illustrates the UV/visible absorption spectra of unmodified ON1 (5'pTGTTTGGC) and ON1-5'pyrene in 50% v/vacetonitrile.

Figure 28 illustrates the UV/visible absorption spectra of unmodified ON1 (5'pTGTTTGGC) and ON1-5' Np in 50% v/vacetonitrile.

Figure 29 illustrates the emission spectra of the SP-3 split-probe system in 80% TFE/Tris buffer.

Figure 30 illustrates the emission spectra of the SP-38 split-probe system compared with those for the 5' Pryene oligo and 5'Pryene oligo+ target DNA, the spectra being recorded in 80% TFE/Tris buffer.

Figure 31 illustrates the emission spectra of the SP-2 split-probe system compared with those for the ON2-3' Pryene oligo, the spectra being recorded in 80% TFE/Tris buffer.

DOUGLAS et al.
Appl. No. 10/554,266
July 17, 2009

Figure 32 illustrates the emissions spectra for a construct based on the SP-19 system but in which one of the probes contained 3 LNE residues, the spectra being recorded in 80% TFE/Tris buffer.

Figure 33 illustrates the emission spectra for a construct based on the SP-19 system but in which one of the probes contained 3 LNE residues and had a mismatch for the target, the spectra being recorded in 80% TFE/Tris buffer.